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## REPORT OF THE WORKING GROUP OF HARMFUL ALGAL BLOOM DYNAMICS (WGHABD)

4–7 APRIL 2005

FLØDEVIGEN, NORWAY



International Council for the Exploration of the Sea  
Conseil International pour l'Exploration de la Mer

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## 1 Welcome and opening of the meeting

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The ICES-IOC Working Group of Harmful Algal Bloom Dynamics meeting was hosted by the Havforskningstitut Flødevigen, Flødevigen Norway from 4–7 April 2005. Twenty-four scientists from fourteen countries participated. The list of participants is presented in Annex 1. The meeting agenda is presented in Annex 2.

The meeting was opened by Einar Dahl from the Havforskningstitut Flødevigen on the 4 April 2005 and the participants were introduced with respect to their names, institute, national affiliation and fields of expertise. The agenda was agreed and Joe Silke elected as Rapporteur.

Being a joint ICES-IOC working group, the IOC in most years announces the possibility for its Member Countries outside the ICES area to attend WGHABD. In 2005 the IOC made the decision to support the Inter-comparison Workshop in lieu of the WG meeting.

The Chair outlined the comments and review from the ICES Oceanography Committee relating to the WGHABD report from the 2004 meeting. The committee felt the report was well organized, informative and the meeting well attended. The resubmission of the resolution for a workshop on new and classic techniques for the determination of numerical abundance and bio-volume of HAB-species in Sweden, August 2005 was supported and encouraged. A joint Theme Session between WGHABD and WGPBI at ICES ASC in 2006 titled “Harmful Algal Bloom Dynamics: Validation of Model Predictions (possibilities and limitations) and status on coupled physical-biological process knowledge” was approved. There was discussion of the low attendance at the ICES WGPE, and how to improve attendance. It was stressed that WGHABD and WGPE have entirely different mandates, as well as researchers that would attend each meeting. WGHABD is also co-sponsored by IOC. It was also felt that if one WG was successful, it would not be wise to compromise it. Terms of reference for WGPE were reviewed and some modified, deleted or revised with hopes of improving attendance.

ICES had changed its policy on invited members to ICES WG meetings. Prior to the 2005 meetings, it was necessary to be identified as an appointed member from each ICES member country in order to attend meetings of a particular WG. This policy was changed at the 2004 Annual Science Conference and it is now possible for Chairs of each WG to invite people who are not official appointees to a WG but would contribute to the discussions and ToRs.

The Terms of Reference for 2005 were reviewed and adopted. The Term of Reference concerning preparation of data and summarizing the distribution and number of harmful algal blooms in the North Sea and identification of any trends over recent decades in the occurrence of these blooms for input to the Regional Ecosystem Study Group for the North Sea in 2006 for the period 2000–2004 was revised. The new Term of reference is to prepare the data for the period 1984 to 2004 (where available), and submit the data to the secure REGNS website in excel spreadsheet format in preparation for the REGNS Integrated Assessment Workshop to be held from 9–11 May 2005. The data should be averaged and presented in ICES grid spatial scale, indicating where no observations have been recorded.

## 2 Terms of reference

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At the 91<sup>st</sup> Statutory Meeting (2004), Vigo, Spain, the Council approved the WGHABD (2004) Terms of References (C. Res. 2C03).

The **ICES-IOC Working Group on Harmful Algal Bloom Dynamics** [WGHABD] (Chair J. L. Martin, Canada) will meet in Flødevigen, Norway, from 4–7 April 2005 to:

- a) Review the dynamics of toxin producing phytoplankton and associated toxins in shellfish, related to phytoplankton abundance, and phytoplankton community structure with references to HAB population dynamics. In 2005 the focus will be toxin producing phytoplankton and associated toxins in shellfish
- b) Consider the status of knowledge concerning biologically active specific chemicals, their chemical nature, presence and production in algae and their effects on individuals and population dynamics, as well as their impacts on ecosystems
- c) Discuss new findings that pertain to HAB dynamics, and define the main processes
- d) Review plans for the proposed Workshop on New and Classic Techniques for the determination of numerical abundance and biovolume of HAB-species
- e) Prepare data on the distribution and number of harmful algal blooms in the North Sea for the period 1984-2004, (where available), and submit the data to the secure REGNS website in excel spreadsheet format in preparation for the REGNS Integrated Assessment Workshop to be held from 9-11 May 2005. The data should be averaged and presented in ICES grid spatial scale
- f) Collate and assess National reports and update the decadal mapping of harmful algal events for the IOC/ICES harmful algal database, HAE-DAT
- g) Review progress in computerized production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years
- h) Propose types of analyses that should be performed using the IOC-ICES HAE-DAT dataset and identify problems and gaps in this dataset that must be rectified before the analyses can be conducted

### 3 Summary and conclusions

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Techniques for analysis and prediction of population dynamics of HABs are not well developed and measures of species-specific growth rates and mortality rates are very difficult. Monitoring is an important aspect of HAB research and the WG needs to interact with monitoring programme designs and data interpretation. For example, more environmental data is often needed and sampling should be rationalised with local hydrography such as mixed layer depth, circulation patterns, frontal dynamics, etc. Historical data and time series data are important in looking for historical occurrences of HABs. Increase and decrease in population size is important to bloom dynamics and modelling HABs.

The importance of the WG approach and focus on population dynamics of specific HAB species and not on phytoplankton ecology in general was emphasised. The economic, resource and environmental effects of HABs are included within the WGHABD. In addition, often phytoplankton ecology models are usually biomass, nutrient, and carbon cycling and in many cases cannot define, explain or predict HAB dynamics. In the past we have had joint meetings with SSO and modellers to try and incorporate physics and HAB dynamics into the models.

The WG felt that the existing ToRs were related and important to dynamics.

**Term of Reference a:** Review the dynamics of toxin producing phytoplankton and associated toxins in shellfish, related to phytoplankton abundance, and phytoplankton community structure with references to HAB population dynamics. In 2005 the focus will be toxin producing phytoplankton and associated toxins in shellfish

Five presentations were made presenting data from Canada, Norway, Scotland, Spain, and the US. It was stressed that action levels for phytoplankton numbers and related shellfish toxicity are country and location specific and in some countries are not reliable. DSP toxicity in mussels can change depending on phytoplankton composition. It was emphasized that regular phytoplankton sampling is important. There is also concern in some countries as non-essential mouse bioassays are no longer allowed.

There was discussion on action levels for closing areas to harvesting as a result of unsafe levels of toxins. A Joint FAO/IOC/WHO Ad Hoc Expert Consultation for *Codex Alimentarius* on Biotoxins in Bivalve mollusks meeting was held recently and there are indications that levels for some toxins may change.

**Term of Reference b:** Consider the status of knowledge concerning biologically active specific chemicals, their chemical nature, presence and production in algae and their effects on individuals and population dynamics, as well as their impacts on ecosystems

A comprehensive overview of the status of knowledge on marine toxins [including secondary amino acids (e.g., domoic acid), purine derivatives (e.g., saxitoxins), cyclic imines (e.g., spirulides, gymnodimine), and polyether toxins (e.g., okadaic acid), macrocyclic (e.g., pectenotoxins) and ladder-frame type (e.g., brevetoxins, yessotoxins)] was presented.

A presentation on allelopathy and cyanobacteria described the findings that both toxic (*Nodularia spumigena*) and non-toxic Baltic Sea cyanobacteria (*Aphanizomenon* sp. and *Anabaena* sp.) decrease growth in cryptophytes and diatoms.

Marine toxins can be influenced by: environmental factors; molecular logic" dictating that secondary metabolites have some adaptive significance; chemical-ecological interactions in eukaryotic microalgae being diverse, complex, highly targeted and often cryptic; understanding that not all behavioural responses to chemical cues are adaptive, (i.e., evolutionarily significant).

**Term of Reference c:** Discuss new findings that pertain to HAB dynamics, and define the main processes

Six presentations were made featuring: the first record of *Dinophysis sacculus* from the Danish and Scandinavian coastal waters in 2004; modelling *Chattonella* spp. in the North Sea and Skagerrak; correlations between eutrophication and chlorophyll *a*, *Phaeocystis*, *Noctiluca* and raphidophytes; satellite detection and model prediction of *Phaeocystis* blooms; modelling vegetative growth, gamete production and encystment of dinoflagellates in batch cultures; and real time monitoring of HABs using a moored vertical profiling system.

Discussions that arose from the presentations included: scientific and management questions requiring answers from modelling; the effects of turbulence on growth rates, information required from both the modellers and biologists; the predictive capabilities of the models; knowledge and technical gaps impeding progress towards the quantification of phytoplankton.

**Term of Reference d:** Review plans for the proposed Workshop on New and Classic Techniques for the determination of numerical abundance and bio-volume of HAB-species

As it was not possible to secure funding for the Workshop in 2004, a decision was made (and approved) to resubmit the resolution to ICES with the intention of holding the Workshop in August 2005. The objectives for the Workshop will be to compare traditional methods of counting HABs using microscopic, molecular and other new techniques. It was emphasized that this Workshop would be an inter-comparison exercise, as opposed to method development and would be restricted to approximately 24 participants who are currently using the identified methods.

**Term of Reference e:** Prepare data on the distribution and number of harmful algal blooms in the North Sea for the period 1984–2004, (where available), and submit the data to the secure REGNS website in excel spreadsheet format in preparation for the REGNS Integrated Assessment Workshop to be held from 9–11 May 2005. The data should be averaged and presented in ICES grid spatial scale.

The subgroup identified that there is a requirement to integrate plankton, survey, fish and marine mammal/seabird databases as a prerequisite for the development of the ecosystem approach to integrated advice. The ICES initiative has been to form the Regional Ecosystem Study Group for the North Sea REGNS.

A list of toxic species in the North Sea is being prepared with guideline levels for the potential to cause harmful effects.

**Term of Reference f:** Collate and assess National reports and update the decadal mapping of harmful algal events for the IOC/ICES harmful algal database, HAE-DAT

National reports were presented for Belgium, Canada, Denmark, Estonia, France, Germany, Ireland, Latvia, The Netherlands, Norway, Poland, Portugal, Spain, Sweden, the U.K., and the U.S.A. Maps were updated for inclusion to the decadal maps. Information for the database was provided in the required format.

**Term of Reference g:** review progress in computerized production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years

Decadal maps are currently being updated manually. A new Decadal maps product which uses both ArcView and Flash softwares, and allows updating of maps from a MySQL database is being explored. The use of the MySQL database both in the new HAE-DAT format and in the new decadal maps will open future technical options for linking these two datasets that will be studied during this year. The capability of linking the maps has been and continues to be extended to additional countries. Most ICES member countries have provided divisions of coastlines and coordinates to enable the linkages.

**Term of Reference h:** Propose types of analyses that should be performed using the IOC-ICES HAE-DAT dataset and identify problems and gaps in this dataset that must be rectified before the analyses can be conducted.

At present, information is entered manually into the HAE-DAT dataset (which is in Access97 format). By next year there will be an electronic format (with the same information as previous forms) available for submission directly into the database. Monica Lion (IOC-IEO-SCCHA, Vigo, Spain) presented a list of potential problems for the conversion of all the old historical records into the new form. Designated country delegates will continue to go through these old records and identify discrepancies.

Although HAE-DAT has been underutilized in the past, it was used to generate economic loss information and display fishery closures. WGSaEM will be given a subset of “clean” data for analyses and suggestions for output.

## 4 Term of Reference A

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Review the dynamics of toxin producing phytoplankton and associated toxins in shellfish, related to phytoplankton abundance, and phytoplankton community structure with references to HAB population dynamics. In 2005 the focus will be toxin producing phytoplankton and associated toxins in shellfish

Presentations were made by Einar Dahl, Beatriz Reguera, Eileen Bresnan, Don Anderson, and Jennifer Martin. The presentations differed in scope, as some were based on monitoring data, others on research cruises and still others on historical analysis. No major milestones or commonalities were identified, other than the reliance of *A. fundyense* on cyst germination for bloom initiation, as described for both the Bay of Fundy and the Gulf of Maine. Progress was satisfactory. A future term of reference should be similar, but focused on other HAB species



or syndromes, such as DSP as well as identifying new remote sensing techniques, results from new sensors and algorithms as well as validation procedures for HAB observations.

#### 4.1 Toxin producing phytoplankton and toxins in shellfish - experience from Norway

Einar Dahl summarized protocols in Norway for establishing opening and closures of shellfish harvesting areas.

Since 1992, regular monitoring of algal toxins in shellfish has been performed along the Norwegian coast from March to October each year. The purpose is to advise people on risks associated with eating wild mussels. The monitoring has two elements, 1) monitoring for potentially toxic algae and 2) analyses of algal toxins in shellfish. When monitoring begins each year, toxin content in mussels is analysed by chemical methods at all monitoring stations to determine the status of toxicity in mussels along the coast. Concurrently, water samples and net hauls are collected for determination of density of potentially toxic algae. After the initiation of monitoring each year, advice based on weekly data on presence of toxic algae from water samples is provided to the general public. For selected potentially toxic algae, numbers have been identified to act as indicator levels at which warnings are issued. For example, when these levels are exceeded in an area, the risks for accumulation of algal toxins is considered to be high and people are advised not to harvest and consume wild mussels from that particular area. It should be stressed that these warning levels are specific for Norwegian coastal waters and based on long-term experience, knowledge pertaining to hydrodynamic conditions and annual phytoplankton cycles along the coast. Warning levels are used to "close" areas for harvesting of wild mussels, and an area is not "opened" again until toxin analyses in shellfish results in levels of toxins below quarantine levels.

#### 4.2 Can we rely on triggering levels of cell concentrations with *Dinophysis* spp.?

Beatriz Reguera summarized work on cautions and guidelines in relation to triggering cell densities of *Dinophysis* spp. and identifying action levels.

Cell concentrations of potentially toxic planktonic microalgae have been used as a tool to trigger increasing or even initiation of toxin analyses in areas with traditional shellfish exploitations or non commercial harvesting of shellfish (Anderson *et al.*, 2001; Todd, 2004). This practice may prove useful in the case of toxic species that are seasonal, develop *in situ*, and need to reach high cell numbers to cause harmful effects. Nevertheless, the use of cell concentrations as a trigger to initiate analyses of toxins in shellfish cannot be considered to be a generally good practice to protect the public's health and to regulate shellfish marketing. This is especially so in the case of toxic microalgae, such as for some species of the genus *Dinophysis* that are the main cause for closures of bivalve harvesting in the Atlantic coasts of Europe and: i) are permanently present in plankton populations; ii) often constitute a small percentage of the global phytoplankton population, and iii) can render shellfish unsuitable for human consumption at very low cell concentrations ( $1-2 \times 10^2$  cell L<sup>-1</sup>). In this document we provide several reasons to caution against or criticize the use of a "*Dinophysis* index" as a trigger to initiate DSP toxins analyses in shellfish growing areas:

- 1) the very high spatial (m) and temporal (d) variability of cell distributions. Conventional samplings are inadequate

The distribution of *Dinophysis* spp. and other swimming dinoflagellates is extremely variable in time and space. Patchiness results from physical-biological interactions such as the aggregation of dinoflagellate cells in the pycnocline, the persistence of swimming organisms in convergent hydrographic cells, transient retention features, etc. Furthermore, physical advective processes such as those

observed during wind reversal, downwelling events, etc. can cause the accumulation of high concentrations of toxigenic cells in time scales of 1–2 days, between two consecutive phytoplankton and/or toxin monitoring samplings (Reguera *et al.*, 1993; Morono *et al.*, 2000). During an intensive 24 h-sampling study at a fixed station, differences over one order of magnitude in *Dinophysis* concentrations over the whole water column were observed (Reguera *et al.*, 2003). There is no possible monitoring programme in the world with sufficient coverage of stations and frequency of sampling to provide, based only on cell counts, an “operational” forecast of the distribution of potentially toxic cells that can become accessible to bivalve feeders on following days. Nevertheless, these limitations can be overcome if the distribution of cells is complemented with knowledge on local hydrodynamics and experience gained from previous toxic events.

2) high variability of toxin content per cell

So far, no one has been successful in establishing *Dinophysis* spp. in culture. Chemical analyses to determine toxin profiles and toxin content per cell have been performed either on net-haul concentrates or on single cell isolates from natural populations. Available data show a high variability (up to 1-2 orders of magnitude) in toxin content per cell whereas the toxin profile in a given location seems to be a more stable feature (Lee *et al.*, 1989; Blanco *et al.*, 1995, 2000; Andersen *et al.*, 1996; Fernández *et al.*, 2001, 2002; Fernández-Puente *et al.*, 2004; MacKenzie *et al.*, 2005). Data for each location are still scattered and a more systematic monitoring on toxin content per cell would be needed to advance knowledge on this topic.

3) absolute concentrations of cells can be meaningless if they are not related to concentrations of other accompanying organisms

Since the late 1980s, Sampayo *et al.* (1990) observed that for similar concentrations of *Dinophysis* spp. in samples, there was an inverse relationship between the levels of toxin found in mussels and the total concentration of phytoplankton, (i.e., toxin content in shellfish depended on the ratio between toxic phytoplankton cells and the global phytoplankton population). Further studies of Blanco *et al.* (1995, 1997) showed the importance of intrinsic factors linked to bivalve physiologies and developed kinetic models that took into account the environmental conditions (temperature, salinity, stability), and the quantity and quality of food available (chlorophyll concentration, and seston). The concentration of toxigenic cells and the toxin content per cell were important parameters introduced in these kinetic models of intoxication and detoxification. Blanco *et al.* (1997) introduced a new parameter, the “toxic quality” of the food, by analogy with the “food quality” term commonly employed in assimilation models for bivalves. Dahl and Johannessen (2001) recommended the use of the ratio between *Dinophysis acuta* concentration and the chlorophyll content as a better index to predict DSP events.

4) toxicity in shellfish in the absence of toxic species

After severe DSP outbreaks caused by proliferations of *Dinophysis acuta* in late autumn, DSP toxin content above regulatory levels can be observed in mussels long after *Dinophysis* cells have disappeared from the water column (see Spain-Galicia and Norway National Reports in this ICES report). In these cases, the end of harvesting closures depends more on the onset of the diatom spring blooms (that will provide abundant non-toxic food to shellfish) than on reported concentrations of toxic dinoflagellates in the water.

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### 4.3 *Alexandrium* species dynamics in Scottish waters

Eileen Bresnan presented results from the UK experiences.

Monitoring for paralytic shellfish poisoning began in the UK in 1968 in response to a PSP outbreak in the North East of England. Monitoring was initially confined to the NE of England from the Firth of Forth to the Humber Estuary. Monitoring was extended around the Scottish coast during the 1990s. Analysis of data from the monitoring programme (in fulfilment of 91/491/EEC) has shown PSP to be detected in *Mytilus edulis* from most sites around the Scottish coast. One exception has been the Loch Linnhe area on the west coast of Scotland where PSP has not been detected. PSP concentrations have decreased from  $>1000 \mu\text{g STX eq. } 100\text{g}^{-1}$  to concentrations below the closure limit of  $< 80 \text{ } 1000 \mu\text{g STX eq. } 100\text{g}^{-1}$ .

Monitoring for toxin producing phytoplankton species began in Scotland in 1995. *Alexandrium* spp. cells are routinely identified to genus level only within this programme. Little work has been done on speciation but analysis from specific studies have shown *A. tamarense*, *A. ostentfeldii* and *A. minutum* to be present.

Cyst surveys have shown *Alexandrium* cysts to be present along the east coast, where *Alexandrium* cells and high levels of the PSP toxin are often detected. Analysis of the data shows a good correlation between the presence of *Alexandrium* spp. in the water column and PSP toxin accumulation in *M. edulis* tissue. However the dynamics of *Alexandrium* spp. blooms within Scottish waters show large inter-annual variability. *Alexandrium* cell densities along the east coast of Scotland are much reduced during May and June while mixed blooms are observed in July and August.

### 4.4 Gulf of Maine *Alexandrium* dynamics

To support discussions on the bloom dynamics of PSP-related organisms, Don Anderson gave a presentation on *Alexandrium* bloom dynamics in the Gulf of Maine, emphasizing the role of life cycle dynamics, and physical forcings in the patterns of toxicity.

A conceptual model of *A. fundyense* dynamics in the Gulf of Maine was proposed, described in more detail in Anderson *et al.*, (in press) and McGillicuddy *et al.*, (in press). This model begins with a large-scale cyst map that includes two major seedbeds – one in the Bay of Fundy (BOF) near Grand Manan Island, and the other offshore of Casco and Penobscot Bays. Cysts germinate from these locations and populate the surface waters in those areas, leading to blooms that are greatly influenced by ambient nutrient concentrations, temperatures, and hydrography.

Outflow from the BOF will carry cells into the Maine Coastal Current system via its eastern branch, called the Eastern Maine Coastal Current (EMCC). This linkage has been depicted in surveys conducted by Martin and White (1988) and Townsend *et al.* (2001). As hypothesized by the latter authors, *A. fundyense* cells that enter the EMCC near the BOF do not initially flourish, due to the deep mixing and high turbulence of that water mass. However, as the cells are transported to the west, the water stratifies and allows growth. Model simulations of this *A. fundyense* population that include a nutrient dependence (McGillicuddy *et al.*, in press) show nutrient limitation at the western edge of the EMCC in the mid-summer months. Based on laboratory studies, nutrient limitation can result in resting cyst formation. Significant offshore cyst accumulations are found in the general area where model results suggest nutrient limitation will occur (McGillicuddy *et al.*, in press), and where Townsend *et al.* (2001) showed abundant populations of *A. fundyense* during large-scale surveys.

The *A. fundyense* populations that cause PSP problems in the western Gulf of Maine (WGOM) region have two possible origins. One is from motile cells delivered to the near-shore waters of the WGOM from the EMCC. The other is from the germination of cysts from

both the inshore and offshore cyst seedbeds that have been mapped out in that region (Anderson *et al.*, in press).

The ultimate scenario is thus of cysts that germinate within the BOF seedbed, causing localized, recurrent blooms to the east of Grand Manan Island that are self-seeding as well as propagatory in nature, supplying cells that populate the EMCC. Some EMCC cells are entrained into western Maine waters, while others eventually deposit cysts offshore of Penobscot and Casco Bays. In subsequent years, these cysts serve as a seed population for the western Maine blooms that are transported to the south and west by the WMCC, causing toxicity along the coasts of western Maine, New Hampshire, and Massachusetts before they are either lost due to mortality or advected out of the region. Without the localized, incubator (and self-seeding) characteristic of the eddy system near Grand Manan Island, one would expect *A. fundyense* populations in the Gulf of Maine to diminish through time and the PSP problem to disappear. Since PSP has been a persistent problem in the region for a century or more argues for the effectiveness and stability of the mechanisms described here.

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## 4.5 Trends in *Alexandrium fundyense* cell densities in the Bay of Fundy, eastern Canada

To support further discussion on bloom dynamics and trends in HAB populations Jennifer Martin presented results from trend analyses for *Alexandrium* populations.

Historical data was examined to characterize *Alexandrium fundyense* blooms and to explore the feasibility of bloom forecasting. *A. fundyense* abundance has been measured at four locations in the Bay of Fundy at weekly to monthly intervals since 1988. The date that *A. fundyense* first appeared varied from day of the year 105 to 179. Between stations, the mean and median dates of the first appearance of *A. fundyense* varied by only a few days. Overall, the mean (median) date of the first appearance was day 136 (134). The null hypothesis that the date of the first appearance varies randomly from year to year could not be rejected ( $\alpha=0.05$ ) by a two-sided runs test. The date of the maximum cell concentration varied, between stations and years, by about 30 days. Maximum cell concentrations occurred earliest at the inshore estuarine station (day 172–175) and latest offshore (day 197–203). The annual maximum concentration of *A. fundyense* varied among stations by about three orders of mag-

nitude and the median value differed from offshore to inshore by about two orders of magnitude. The total annual duration of the *A. fundyense* presence ranged from 50 to 200 days and had a mean of 120 days while the duration of the bloom containing the annual maximum concentration varied from 10 to 160 days. The temporal character of the *A. fundyense* bloom also varied between years and stations with the number of blooms or abundance pulses varying from 1 to 3 per year.

Although there have been annual closures of shellfish harvesting areas in the southwest New Brunswick area for many years, the duration of the closures and the intensity of the toxin levels varies on inter-annual and decadal time scales. Until recently, the shellfish industry has not suffered from extended periods of closure since the major periods of PSP and *A. fundyense* blooms in the late 1970s and early 1980s. However, in 2003, *A. fundyense* cell concentrations reached levels as high as  $8.0 \times 10^5$  cells  $L^{-1}$ , the highest concentrations observed since the early 1980s. In 2004, cell counts were as high as  $10^6$  cells  $L^{-1}$ , the highest observed since standard phytoplankton sampling in the area began. These high cell numbers resulted in higher accumulation levels of toxin in shellfish resulting in longer retention times of the toxins in tissues at levels above regulatory limits. In the future, further analyses concerning the PSP toxicity and phytoplankton count data will be pursued to better estimate potential impacts.

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Following the presentations, a number of related issues and concerns were discussed. It was stressed that when determining action levels for phytoplankton, in some regions there is good historical information, which is necessary in identifying levels. In addition the variability of toxin content per cell is very high and the amount of non toxic seston present in the waters is important. By applying a precautionary principal the usefulness of algal counts is justified to give the public

As part of the population dynamics of HABs, it is important to understand life cycles. One important part is the “germling”. Germling is a neutral descriptor of the cell type emerging from the resting cyst (hypnozygote) in the sexual life history of dinoflagellates. The biflagellated cell that hatches from a hypnozygote is commonly designated as a “planomeiozyte”. This designation is deduced from analogous knowledge derived mainly from the life history of higher plants. Meiosis has not been thoroughly explored in dinoflagellates. It remains unclear whether or not dinoflagellates have a normal two-step meiosis; a one-step meiosis has been reported for at least one *Alexandrium* species. As there is no definitive knowledge regarding exactly when meiosis occurs in the life history of dinoflagellates, we recommend the use the neutral term “germling” for the cell with two longitudinal flagellae hatching from a hypnozygote.

A sub group met and discussed the feasibility of identifying specific phytoplankton numbers for specific toxins. The following is a summary of their discussion but pertains mostly to Spain and Dinophysis.

According to EU Directives (91/492/EC, 91/493/EC, 2002/225/EC, 2002/226/EC), shellfish harvesting and placing on the market is based on levels of toxins in shellfish flesh. Monitoring

potentially toxic phytoplankton species is also specified in the same directives. Thus, potentially toxic phytoplankton levels would never be the decisive parameter to control marketing, but it is recognized as extremely useful complementary information to the analyses for the following reasons:

- The occurrence of potentially toxic species can indicate the potential for future shellfish intoxication;
- Phytoplankton levels combined with knowledge on the local hydrodynamics are the basis for expert judgment and management of the situation (change of monitoring intensity, guidance to target specific toxins, expected location and duration of opening and closures of shellfish harvesting.
- Acquisition of long term databases on harmful phytoplankton species and on global phytoplankton populations are needed to test hypothesis on causes of harmful algae events.

Fixed levels of phytoplankton should not be uniformly used for management purposes in all ICES countries. The use of phytoplankton levels to modify action plans has to be based on: i) local knowledge of the harmful algal bloom dynamics; ii) the species-specific kinetics of intoxication and detoxification of the exploited shellfish resources, and iii) the experience acquired after years of information gathering and assessment.

On a regional basis, levels of phytoplankton influencing management of shellfish resources should be open to revision year to year in accordance with new findings and experience.

At present there are only a couple of countries that are using triggering levels - where the toxicity and the toxicity profiles of the shellfish are very well defined and there may be some utility in this approach in these countries.

However it was emphasized that:

- spatial (1m) and temporal (1day) variability can be very high: conventional sampling is often insufficient;
- toxin concentration varies between individual cells (> one order of magnitude);
- results do not mean much if not related to accompanying seston;
- large differences in cell densities are often observed between offshore and inshore sampling locations.

It was agreed that this ToR be continued but addressing other key species.

## **5 Term of Reference B**

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Consider the status of knowledge concerning biologically active specific chemicals, their chemical nature, presence and production in algae and their effects on individuals and population dynamics, as well as their impacts on ecosystems.

### **5.1 Ecological Chemistry and Effects of Phycotoxins in Marine Ecosystems**

Allan Cembella presented the following comprehensive summary of the state of knowledge on chemistry and phycotoxin effects.

Among the thousands of species of marine microalgae, several dozen produce highly potent natural toxins that profoundly affect the health of marine ecosystems and human consumers of seafood. These toxins of algal origin (phycotoxins) are most often synthesized by marine flagellates (particularly dinoflagellates), but certain diatoms and cyanobacteria are also implicated. Phycotoxins are responsible for marine faunal mortalities and intoxication syndromes linked to seafood consumption (ciguatera, DSP, NSP) from tropical to polar latitudes. In general,

these toxins can be divided into several major structural sub-classes – secondary amino acids (e.g., domoic acid), purine derivatives (e.g., saxitoxins), cyclic imines (e.g., spirolides, gymnodimine), and polyether toxins. The various polyether toxins may be described as linear (e.g., okadaic acid), macrocyclic (e.g., pectenotoxins) and ladder-frame type (e.g., brevetoxins, yessotoxins). They are influenced by the following:

- environmental factors;
- “molecular logic” dictating that these specific secondary metabolites have some adaptive significance;
- chemical-ecological interactions in eukaryotic microalgae being diverse, complex, highly targeted and often cryptic;
- understanding that not all behavioural responses to chemical cues are adaptive, i.e., evolutionarily significant.

Although it is still not possible to definitively ascribe a functional role to phycotoxins, and gene regulation of toxin production remains poorly understood, hypotheses concerning their evolutionary significance and biogeographical distribution must be addressed. Many of these challenges can be approached via application of novel analytical chemistry (e.g., LC-MS/MS) and gene technologies. Phycotoxins are defined as biologically active natural products, which can affect receptors and metabolic processes in a variety of ways – as Na-channel blockers, Ca-channel activators, glutamate agonists or protein phosphatase inhibitors. The mode of pharmacological action of some phycotoxins, such as the “fast-acting toxins” (e.g., gymnodimine, spirolides) is poorly understood and human health significance is unknown. Despite recent advances in structural elucidation and the determination of biosynthetic pathways of phycotoxins, relatively little is known about the structural/functional relationships of these secondary metabolites.

In spite of the fact that phycotoxins are chemically diverse, a number of generalizations can be advanced regarding their distribution and characteristics:

- distribution is phylogenetically diverse (occurring among both prokaryotes and eukaryotes);
- biosynthesis is inconsistent among strains and species within a genus;
- toxin-producers are primarily free-living marine photoautotrophs;
- toxin content is subject to environmental modification but composition is rather stable, and presumably genetically determined;
- cellular growth rate ( $\mu$ ) and toxin production are usually positively correlated, i.e., the toxins are not “stress” metabolites (domoic acid production by certain diatoms is an important exception);
- many phycotoxins are potent ion-channel effectors or enzyme regulators.

A key caveat is that the toxic properties of these compounds have typically been defined with respect to the pharmacological properties and mode of action in whole-animal mammalian bioassays and in tissue cultures. Thus, the known toxicological properties that have been observed and quantified may or may not be related to the functional significance of these substances to the producing organisms and in the general ecosystem. For example, although it was initially suspected that the induction of retrograde swimming (avoidance) behaviour in the tintinnid *Favella* was related to the PSP toxin content of the potential prey species *A. ostentfeldii*, and later to spirolide toxins when their presence was confirmed in the prey cells, we now believe that these toxins are not responsible for the observed allelochemical interactions. Recent work on the “allelochemical phenotype” of multiple isolates of *A. tamarensense* from the North Sea exposed to the phagotrophic dinoflagellate *Oxyrrhis* showed no correlation between the PSP toxin content, related toxicity, and the magnitude of effects on the protistan grazer.



There is increasing evidence that the functional significance of phycotoxins in marine ecosystems can be addressed from the perspective of chemical ecology. In this sense, chemical ecology refers to “Darwinian chemistry” – the relationship between structure and function of metabolites and their interactions among organisms in the environment, controlling coexistence and co-evolution of species. Based upon their high specific potency, various hypotheses relating to their in situ ecological significance of phycotoxins have been advanced. These hypotheses remain highly speculative, but they include putative roles as allelopathic agents against species competing for a common ecological niche, pheromones (sexual attractants), or as chemical defence mechanisms against predators. Other hypothetical roles include the functions of: UV photoprotectants, life history modulators, enzyme regulators, membrane transport effectors, anti-bio-fouling metabolites, and internal nutrient reserves.

The incorporation of chemical ecological rate processes and effects into biological-chemical-physical coupled models should be considered. In many bloom dynamic models, chemical variables are typically limited to “nutrients”, and in most cases data sets are restricted to measurement of ambient concentrations and the use of rate constants for uptake and assimilation of dissolved inorganic macronutrients. Parameterization of models of harmful algal bloom dynamics may also require detailed knowledge of the functional role of phycotoxins and other marine bioactive substances. At least in theory, if not in practice (this remains to be fully investigated), marine bio-actives may contribute to either the growth or loss terms via effects on inter-species competition for limiting resources, grazing inhibition, predator mortality and morbidity, and water conditioning by production of “growth promoting” substances. Grazing interactions and phytoplankton growth in pelagic food webs may be mediated by chemical cues governing selectivity and inhibition. This has been referred to by V. Smetacek as a “watery arms race”.

Marine ecological chemistry includes chemical ecology but also natural products and bio-analytical chemistry, classic marine chemistry, eco-toxicology and bio-diagnostics related to the ecological interactions of natural and anthropogenic substances. The strategy for ecological chemistry studies relevant to algal bloom dynamics should be to move beyond a static quantitative and qualitative description of substances in ecological compartments towards a dynamic perspective within which the functional significance of bioactive substances and their interactions are defined and compared.

Comparison of toxin composition among natural dinoflagellate populations typically reveals a high degree of structural polymorphism and the expression of geographically distinct profiles. Nevertheless, recent studies based upon >200 clonal isolates of *Alexandrium tamarense* from bloom populations sampled off the Scottish east coast (Firth of Forth to the Orkney Islands) have revealed a remarkable diversity of “toxin phenotypes” within a given bloom population. At least for *Alexandrium* spp., the toxin spectrum is usually conserved upon transfer and long-term maintenance in clonal culture, and tends to be quite refractory to environmental perturbations - this suggests a strongly defined genetic template. However, the toxin cell quota may vary markedly over the cell cycle and/or culture cycle in response to physiological changes. For example, the metabolic cascade leading to the synthesis of polyether compounds via polyketide biosynthesis, such as the production of DSP toxin derivatives by the benthic dinoflagellate, *Prorocentrum lima*, and spirolide production by the planktonic species, *Alexandrium ostenfeldii*, has been investigated using photoperiod-induced cell synchronisation techniques. Regulation of polyether toxin biosynthesis in the cell cycle is currently being studied at the transcriptional level by differential display of RNA expression. These polyether toxins are constitutively produced - they are not classic “stress” metabolites. Current efforts are focused on establishing the timing and sequence of key cell cycle events involved in the biosynthesis of polyether toxins.

In summary, the role of marine toxins and other bioactive substances may be described as follows:

Synthesis of these compounds is regulated by an interplay between intrinsic cellular functions now possible to measure trace concentrations (sub-picomolar) of phycotoxins directly from seawater matrices and even in individual cells for some types of toxins. Recent evidence from North Sea isolates of *A. tamarense* exhibited no obvious relationship between PSP toxin phenotype, and population genetic markers such as AFLP (amplified fragment length polymorphism) and microsatellites. Gene expression studies involving the construction of “expressed sequence tags” (ESTs) from cultured isolates (to date: *Chrysochromulina polylepis*, *Prorocentrum lima*, *A. ostenfeldii* and *A. tamarense*) subjected to a variety of environmental conditions (of light, temperature, salinity, and nutrients) have already added substantially to our knowledge of the regulation and biosynthesis of certain phycotoxins. Research on the chemical ecology of marine phycotoxins will contribute greatly to understanding their significance in the population dynamics of the producing organisms and the consequent effects on marine trophic webs.

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## 5.2 Allelopathic effects of the Baltic cyanobacteria

Sanna Suikkanen presented the following evidence of allelopathy.

Extracts of both toxic (*Nodularia spumigena*) and non-toxic Baltic Sea cyanobacteria (*Aphanizomenon* sp. and *Anabaena* sp.) have been found to decrease the growth of cryptophytes and diatoms in experimental studies. In a natural plankton community, cyanobacterial extracts also caused a stimulation of other cyanobacteria, nano- and dinoflagellates and chlorophytes. In addition, there are indications of mutual allelopathy between *N. spumigena* and *Aphanizomenon* sp., Nodularin is unlikely to be the cause of allelopathy in *N. spumigena*, although the toxin may be incorporated into phytoplankton cells exposed to it.

The group discussed the transformation of toxins and toxin levels and the fact that a culture should have the same toxin profile and toxicity when tested a year later. It was noted that individual isolates grown at different growth rates can have different toxin profiles and that within cultures cells may no longer be clones. Cyanobacteria have been known to occasionally lose their toxicity when they lose their associated viruses.

## 6 Term of Reference C

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Discuss new findings that pertain to HAB dynamics, and define the main processes.

### 6.1 Modelling of Harmful Algae Blooms. The case of *Chattonella* spp. in the North Sea and Skagerrak

Morten Skogen presented results from modelling *Chattonella* spp. in the North Sea and Skagerrak.

There have been several large blooms of the harmful algae *Chattonella* spp. in the North Sea and Skagerrak during the last several years. Both in 1998 and 2001 the bloom extended towards the south-eastern coast of Norway causing fish mortalities in fish farms in the area. A *Chattonella* module has been implemented in the 3-dimensional coupled physical, chemical and biological ocean model NORWECOM. A sensitivity analysis was performed, and the years 1998–2001 have been modelled using realistic forcing. With the present settings, the

model produces an annual one-month bloom of *Chattonella* in the north-eastern North Sea and Skagerrak starting in late April. The model also confirms that calm conditions are necessary for a bloom of *Chattonella* to occur, and indicates that a turbulence dependent death rate is needed for a proper balance between death and growth rates. It is concluded that further laboratory experiments to investigate turbulence dependent death of *Chattonella* are needed. Due to the salinity dependent growth of *Chattonella*, salinity is important both for triggering and ending blooms in the model. Therefore, a fine resolution model that both resolves salinity profiles and the Norwegian Coastal Current is needed before realistic predictions of *Chattonella* blooms can be done.

The following parameters were incorporated into the model:

Growth rate =	$P_{\max} = 1.6 \times f(S,T)$
Sinking =	-12m/day
Death =	0, $\log_{10}(K_H) < -4$
Death =	10%, $\log_{10}(K_H) = 0$

When the initial run was made on the model using the above settings, there were no blooms in the Skagerrak. However, changing the growth rate to 1.5 and death rate to 1% gave results closer to real events.

Adding wind stress in 1998 before the bloom helped with accuracy as there was a relatively quiet few weeks of calm wind before the bloom. This was similar in 2000 and 2001 when the blooms terminated with the onset of high wind conditions. Information important to running the model includes: changing the slope of the death rate to 0.25 gives closer simulation to real events; the bloom in 1999 came after a short period of relatively calm conditions (~1 week); death rate should be exponential; lab studies of death (turbulence) are necessary to fine-tune the model; salinity dependant growth is important for triggering and ending bloom; there is a need for higher model resolution (horizontal and vertical) for better representation of salinity profiles.

## 6.2 *Dinophysis sacculus* - First record from Danish and Scandinavian coastal waters in August 2004

Per Andersen presented evidence of the first record of *Dinophysis sacculus* in Danish and Scandinavian waters.

Routine monitoring of Danish coastal waters for the occurrence of toxic phytoplankton species has been going on since 1990 as part of the management of the Danish shellfisheries. More than 700 samples are analysed on a yearly basis. For the first time in the monitoring period, the DSP-toxic dinoflagellate *Dinophysis sacculus*, (Figure 6.2.1) was observed in a period of 3 weeks during August 2004 but only in one harvest area in the Isefjord, which is a shallow (depth <10 m), brackish water, semi-enclosed and eutrophicated fjord with connection to the southern part of Kattegat. The observed concentrations of *Dinophysis sacculus* were 100–200 cells L<sup>-1</sup>. Cell length and width was 50–60 µm and 30–35 µm, respectively. *Dinophysis sacculus* co-occurred with the species *Dinophysis acuminata*. Other common *Dinophysis* species in the area such as *D. norvegica*, *D. rotundata* or *D. acuta* were not observed during the *Dinophysis sacculus* bloom. DSP-toxicity in shellfish (*Mytilus edulis*) was not observed during the bloom of *Dinophysis sacculus*. *Dinophysis sacculus* has not been previously reported from Danish or other Scandinavian waters.

*Dinophysis sacculus* is common in the Mediterranean. In the Northern Atlantic the species has been reported to only occur regularly in Galician coastal waters. Furthermore, it has been re-

ported to occur rarely or sporadically (single observations) in only a few other European countries such as the Netherlands, Germany (North Sea) and Ireland.

The source of *Dinophysis sacculus* that initiated the Danish bloom is not known. Further investigations on the taxonomy of *Dinophysis sacculus* using SEM as well as molecular and genetic methods based upon material collected during the reported bloom is on-going in collaboration with scientists from the IOC HAB Centre at the University of Copenhagen.

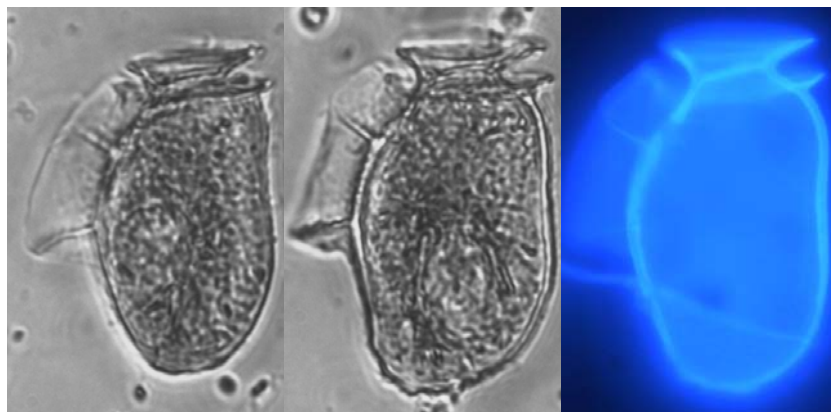


Figure 6.2.1. *Dinophysis sacculus* from the Isefjord, Denmark collected in August 2004.

### 6.3 Correlation between eutrophication and chlorophyll-*a*, *Phaeocystis*, *Noctiluca* and raphidophyte abundance.

Louis Peperzak presented the following presentation as part of new findings.

A quantitative correlation between anthropogenic eutrophication and blooms of HAB species would be an indication that the risk of HABs can be diminished and ecosystem quality can be improved by reducing nitrogen and phosphorus discharges into the sea. Such a correlation forms the basis for certain objectives of OSPAR and the EU Water Framework Directive.

Using >10 year monitoring data from the Dutch part of the North Sea (14 locations) it is shown that *Phaeocystis globosa*, *Noctiluca scintillans*, *Fibrocapsa japonica* and *Heterosigma akashiwo* appear more frequently and at higher concentrations in eutrophic (coastal) than in oligotrophic (off-shore) waters. On the other hand, *Alexandrium* spp. and *Dinophysis* spp. are found more frequently in off-shore than in coastal waters, but the concentrations of these PSP and DSP causing dinoflagellates between these two areas were not significantly different.

Diagrams of chlorophyll *a* and *P. globosa* versus salinity show unusual and unexpected high concentrations of both at two off-shore stations between The Netherlands and England. Using satellite imagery it is hypothesized that the Thames River plume is the source of these enhanced chlorophyll *a* and *P. globosa* concentrations.

### 6.4 Satellite Detection and Model Prediction of *Phaeocystis* Blooms

Louis Peperzak presented a new project that has been initiated in the Netherlands to provide forecasting of *Phaeocystis* blooms.

*Phaeocystis globosa* blooms in The Netherlands can cause damage to the ecosystem and commercial shellfish by producing benthic anoxia. In past years, the Dutch early warning system relied on samples from just one coastal station in which *P. globosa* cells were counted. Integrated Spatial and spectral Characterisation of Harmful Algal blooms in Dutch coastal waters (ISCHA) is a new project that uses chlorophyll *a* maps derived from a satellite spectrometer (MERIS) that are fed into coupled algal growth and transport models. Using five-day weather forecasts the development of the *Phaeocystis* bloom and its transport to coastal areas

that are vulnerable to anoxia are calculated so that local water managers and shellfish growers can be notified in advance with a “HAB-bulletin”.

## 6.5 Modelling vegetative growth, gamete production and encystment of dinoflagellates in batch cultures

Louis Peperzak presented a dinoflagellate growth model.

A model of dinoflagellate growth and encystment was made in which the mitotic cycle (vegetative growth) is coupled quantitatively to the sexual cycle (cyst formation) by having 4 gametes emanate from 1 vegetative cell. Calibrated on literature data of *S. lachrymosa* in this model satisfactorily describes motile cell (vegetative cells and gametes) and cyst development with correlations between log-transformed model and experimental data of  $r^2 = 0.80$  (motile cells) and  $r^2 = 0.94$  (cysts) and with typical maximum rates in the exponential growth phase of  $\mu_{\text{cell}} = 0.55 \text{ day}^{-1}$  (gross vegetative cell rate),  $\mu_{\text{gamete+cell}} = 0.38 \text{ day}^{-1}$  (net motile cell growth rate),  $\epsilon = 0.42 \text{ day}^{-1}$  (encystment rate). All these rates declined in stationary growth phase. A new method for measuring *in situ* encystment rates of dinoflagellate populations with a phased sexual cycle is proposed.

## 6.6 Real time monitoring of high biomass harmful algal blooms (HAB:s) using a mooring with a vertical profiling system in the Skagerrak, NE Atlantic

Bengt Karlson presented the following plans for real time monitoring of high biomass HABs.

Understanding the dynamics of HABs requires the use of several techniques complementing each other (e.g., sampling from research vessels, ferrybox-systems, moorings, remote sensing etc.) Real time monitoring of harmful algal blooms with the necessary vertical resolution for detection of thin layers of phytoplankton is now possible using moorings. The Skagerrak is an area influenced by water from the Baltic and the North Sea. Harmful algal blooms are a major concern in the area for aquaculture and the whole marine ecosystem. The water is mostly stratified and harmful algal blooms sometimes occur in thin layers in the water column. To monitor the development and advection of blooms, a mooring with a profiling multi-parameter device is used. The system also consists of a surface buoy with sensors at 1 m depth and a sea floor mounted acoustic doppler profiler for measurement of current speed and direction as well as waves. The profiling device moves vertically through buoyancy control with a speed of ca 30 cm/s. It is fitted with sensors for chlorophyll *a* fluorescence, turbidity, oxygen, salinity, temperature and nitrate. The vertical resolution is ca 20 cm and profiles are made every three hours. The mooring will be deployed at ca 50 m depth close to the Swedish coast at Måseskär. Reference measurements from research vessels are planned when the mooring is operational in summer 2005.

### 6.6.1 A note on the effect of sunlight on the *in situ* fluorescence from chlorophyll *a*

Part of the standard equipment on most research vessels is a CTD fitted with an instrument for the detection of *in situ* chlorophyll *a* fluorescence. The CTD is used for measuring conductivity, temperature and depth (pressure) and salinity and density of seawater is calculated. The *in situ* fluorometer is used to provide an estimate of the vertical distribution of phytoplankton biomass in the water column. The effect of sunlight on chlorophyll *a* fluorescence was shown with hourly data from the oceanographic mooring “Læsø East” in the Kattegat. Data from a chlorophyll *a* fluorescence sensor mounted at 2 m depth showed a strong diurnal variation. Night time values were often 2–3 times higher than data from mid day. A hypothesis that this was due to vertical migration of phytoplankton was rejected since simultaneous turbidity measurements did not show the same pattern. The effect of sunlight on chlorophyll *a* fluores-

cence is well known for many in the field of optical oceanography and termed photoquenching of chlorophyll. The effect needs to be taken into account when interpreting profiles of chlorophyll *a* fluorescence from different times of day or from different light situations. The effect is strongest close to the surface and may be small below 5–10 m depth.

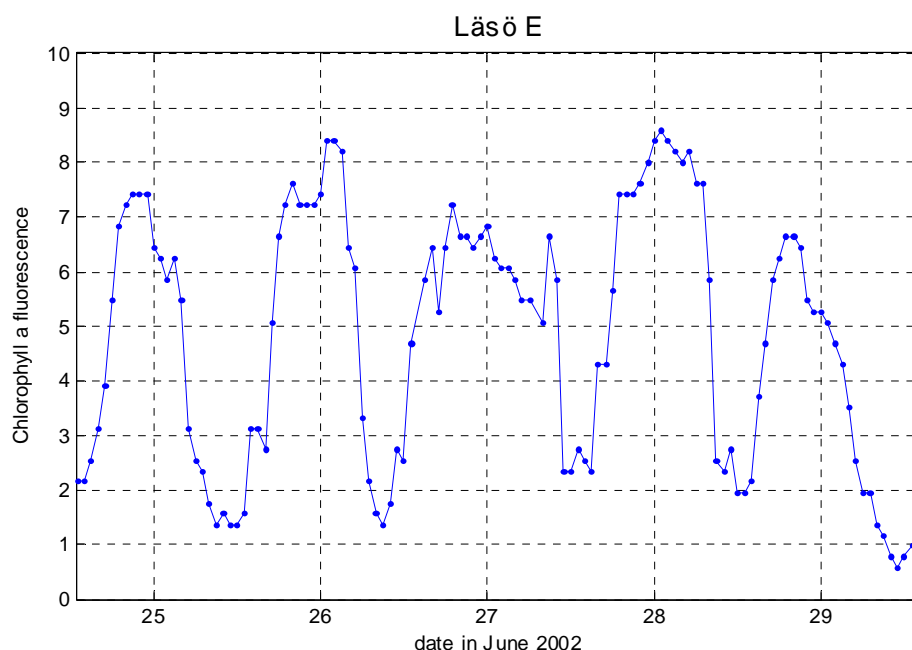


Figure 6.6.1.1. Chlorophyll *a* fluorescence at 2 m depth from the SMHI mooring Läsö East in the Kattegat. The unit on the y-axis is arbitrary. The highest values are around midnight and the lowest around mid-day due to photoquenching.

## 7 Term of Reference D

Review plans for the proposed Workshop on New and Classic Techniques for the determination of numerical abundance and bio-volume of HAB-species

Bengt Karlson presented plans for the joint ICES-IOC Inter-comparison Workshop. Eileen Bresnan (UK) has agreed to be a co-convenor for the workshop with the responsibility for the classic technique methods. The other convenors remain Bengt Karlson (Sweden), Caroline Cusack (Ireland) and Odd Lindahl (Sweden). Although the financial situation for the workshop is not as good as the group had hoped, invitations were sent out in March 2005 to the list of participants that were agreed upon during the WGHABD-meeting in 2004. Requests for other scientists to participate will be considered by the scientific steering committee and a standby list will be maintained.

It was stressed that the workshop is not a training workshop, but an inter-comparison exercise. It will take place at the Kristineberg Marine Research Station (Kristineberg, Sweden) from 22–26 August 2005. Due to the financial situation, participants will probably have to pay for their own travel and accommodations. The scientific steering committee met during the WGHABD meeting and discussed the workshop venue and experimental design. The workshop will focus on the single species, *Alexandrium fundyense*, as all the techniques are able to analyse that particular species quantitatively. This feature can be both a strength and a weakness. In studies of HAB dynamics it is usually important to analyse the whole phytoplankton community but due to the restricted time during the workshop and the large volume of samples to be analysed, it was decided to prioritise and use the single species approach to give a proper statistical experimental design. Bio-volume measurements were given a low priority by

the scientific steering committee since the results from molecular techniques only include cell densities.

Cultures of *Alexandrium fundyense* will be raised and prepared for the purpose of comparing traditional light microscope methods and genetic methods. Equipment including microscopes, coulter counters, DNA equipment and general lab facilities will be made available at Kristineberg.

An invitation list has been prepared and circulated to 26 invitees; a statistician from BioConsult A/S has been assigned the task of setting up experimental setups. The justification for using a single clone of a single species is to facilitate the design of experiments to compare methods. Funding issues have yet to be finalised, but there may be some funds available to assist participants.

## **8 Term of Reference E**

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Prepare data on the distribution and number of harmful algal blooms in the North Sea for the period 1984–2004 (where available), and submit the data to the secure REGNS website in excel spreadsheet format in preparation for the REGNS Integrated Assessment Workshop to be held from 9–11 May 2005. The data should be averaged and presented in ICES grid spatial scale

There is a requirement to integrate plankton, survey, fish and marine mammal/seabird databases as a prerequisite for the development of the ecosystem approach to integrated advice. The ICES initiative Regional Ecosystem Study Group for the North Sea REGNS

The working group discussed the deliverables for the forthcoming REGNS committee in May 2005. Einar Dahl (Norway) will attend the meeting and submit the information from the WGHABD. It was decided to submit two separate tables describing the data available for submission. The strengths and limitations of the available data should also be clarified to the committee.

A table will be prepared to detail the meta data available and list the harmful species in the North Sea (from IOC taxonomic list, inclusion of cyanobacteria in the list is to be discussed by the Danish and Swedish delegates), synonyms, harmful effect etc.

Another table will be assembled to show the datasets available and list the national representative and institution responsible for each dataset. It will also show other parameters that are available for each dataset. The representative for each dataset should be contacted by REGNS if they need additional information.

It was decided that raw data should be sent to the REGNS committee to allow them to do the ‘averaging’. National representatives should stay in contact with holders of datasets to clarify their limitations, etc.

Members of the WGHABD North Sea subgroup will remain in e-mail contact to discuss the outcome of the REGNS meeting the May 2005. An *ad hoc* meeting of this group will be arranged during the upcoming year if necessary.

## **9 Term of Reference F**

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Collate and assess National reports and update the decadal mapping of harmful algal events for the IOC/ICES harmful algal database, HAE-DAT

## 9.1 Belgium

Although Belgium has not had a toxin monitoring programme, there are plans to initiate a programme to measure shellfish toxicity in 2005.

## 9.2 Canada

**Atlantic Coast:** The Newfoundland region did not have any closures due to marine toxins during 2004. In the Gulf of St. Lawrence, there was one area, Miramichi Bay, closed to harvesting for shellfish following levels of 300 µg STX eq. 100 g<sup>-1</sup>. The St. Lawrence Estuary experienced a normal year for closures of shellfish harvesting areas due to PSP and ASP toxins were detected in scallops. The Bay of Fundy had abnormally high concentrations of *Alexandrium fundyense* (and water discolouration in some areas) that resulted in 3 human illnesses as well as prolonged closures of shellfish harvesting areas and mortalities to salmon. Levels of PSP toxins in blue mussels exceeded 18,000 µg STX eq. 100 g<sup>-1</sup> at one salmon aquaculture operation (Beaver Harbour). The major part of the bloom persisted from July through August. A number of shellfish area closures remained in effect from July through December.

**West Coast:** There were a number of closures of shellfish harvesting areas due to unacceptable levels of PSP. Salmon mortalities occurred following a bloom of *Heterosigma* at the following locations: Eeranza Inlet (early June); Bedswell and Clayoquot Sounds (mid June–late July); Kyoquot Sound, Vancouver Island (last week August–mid September); and Broughton Sound (last 3 weeks September). *Dictyocha* was implicated in salmon mortalities in Knight Inlet during the first week of June; *Chattonella* in Queen Charlotte Strait in early October; and *Chaetoceros concavicornis* in the Hoskyn Channel in early–mid April.

## 9.3 Denmark

In Denmark there were no cases of PSP or ASP in 2004. DSP was detected in concentrations above regulatory limits in:

- one production area in the Isefjord in January (causative species - *Dinophysis acuta*)
- three production areas on the east coast of Jutland in January (causative species - *Dinophysis acuta*) and again in one production area in June (probable causative species - *Dinophysis acuminata*)
- one production area in the Limfjord in September (causative species - *Dinophysis acuminata*)

*Alexandrium tamaranense* and *A. ostenfeldii* were observed in low concentrations (max. concentration <200 cells L<sup>-1</sup>)

*Pseudo-nitzschia* spp. were present during the summer of 2004 in the Limfjord as well as in the Kattegat area but in lower concentrations than previous years. No ASP toxicity was observed during the blooms.

A kill of caged fish was observed in the Belt Sea area during an intense bloom (May to the beginning of June - max. concentration >5 million cells L<sup>-1</sup>) of the naked form of the flagellate *Dictyocha speculum*. The bloom was first observed in the pycnocline (depth 5–15 m). Fish kills were observed at the end of the bloom period when the naked form of *Dictyocha speculum* left the pycnocline and entered the surface waters. No mortalities in wild fish were observed during the bloom. *Chattonella* spp. was detected in low concentrations during the spring and early summer.

PSP was observed above the regulatory limit in scallops harvested during the period from September/October 2004 at the West Coast of Greenland (Attu-area). The maximum concentra-



tion was 912  $\mu\text{g STX eq. kg}^{-1}$ . There were no observations on the occurrence of toxic algae for that particular period and area.

#### 9.4 Estonia

Biomasses of cyanobacteria were surprisingly low in the Gulf of Finland along the weekly monitored transect between Tallinn and Helsinki in the Gulf of Finland in the summer of 2004. The weather in the second half of July and in the beginning of August was warm and sunny but the continuous upwelling along the northern coast of Estonia was effective in preventing the development of cyanobacterial blooms. The heavy bloom was formed along the central part of the Gulf of Finland and the surface accumulations were mainly formed by potentially toxic *Nodularia*. This was observed by satellite imagery and by visual observations from research vessels. The measured (in the upper 10 m pooled sample) non-toxic *Aphanizomenon* biomass was as high as 7  $\text{mg L}^{-1}$  in the coastal zone of the Narva Bay (EE-01) on 9 July and the biomass of potentially toxic *Nodularia* rose up to 1.4  $\text{mg L}^{-1}$  in the central part of the Narva Bay (off shore EE-01) on 19 July. Measured *Nodularia* biomasses were comparable with those measured in 1999 and 2002 in the same area. In the beginning of August already a second year a very heavy *Nodularia* bloom was detected in the Väinameri area (EE-07), especially south of Island Hiiumaa in the Kassari Bay. Cyanobacterial biomass was not measured but the surface accumulations were very dense and washed into the shore along a few kilometers. Toxin analyses were not done but a suggestion to close a bay for swimming was made.

#### 9.5 Finland

The intensity of the cyanobacterial blooms in 2004 was between the moderate year 2003 and the high numbers observed in 2002 - the most recent year experiencing exceptionally large blooms. Generally the most extensive cyanobacterial surface accumulations were observed between 25 July and 10 August in the Gulf of Finland, as well as in the Baltic Proper.

The record-breakingly high phosphate levels observed in the surface water masses in the Gulf of Finland in the winter of 2003 to the summer 2004 contributed to the extensive growth of cyanobacteria leading to the high biomasses. However, the cyanobacterial blooms took place later in the year than usual. This was mainly due to the relatively cold early summer that seemed to delay the onset of the blooms. However, in 2004, surface accumulations did not fully reflect the cyanobacteria biomass in the water because the calm periods were relatively short, thus not allowing surface accumulations to emerge.

The cyanobacterial blooms that commenced in early July were dominated by the non-toxic taxon *Aphanizomenon flos-aquae*. The toxic species *Nodularia spumigena* was not abundant until the end of July, mainly due to low water temperatures. The most prominent surface accumulations were found in the middle parts of the Gulf of Finland, although surface accumulations covered some areas in the western part for a longer time period. No notable cyanobacterial blooms were observed in the Gulf of Bothnia.

#### 9.6 Germany

The protocol for routine monitoring of phytoplankton is unchanged since the last report. Toxic algae have been reported repeatedly each year. Species of the genus *Dinophysis* (Dinophyceae), were present, but mussel harvesting was not closed. Other toxic dinoflagellates such as *Alexandrium tamarense* and *A. ostenfeldii* are present each year but in low numbers, not causing PSP problems.

Various *Pseudo-nitzschia* species (Bacillariophyceae) are found regularly in coastal waters, but so far no ASP-intoxications have occurred. Ichthyotoxic members of the Raphidiodiphyceae, *Fibrocapsa japonica* and *Heterosigma akashiwo*, have been present in the last few years but without causing any detected harm as yet.

A benthic euglenoid, *Euglena* sp., is now regularly discolouring large areas of the German tidal flats of the North Sea in Niedersachsen and Schleswig-Holstein. The question arises as to whether this species has been overlooked in the past, as no discolorations were reported earlier or whether it was introduced with aquaculture products.

In the Baltic Sea during 2004 there were no massive developments of potentially toxic cyanobacteria recorded - in contrast to 2003, when beaches had to be closed to the public at various locations due to the massive scum of toxic cyanobacteria, mainly *Nodularia spumigena*.

## 9.7 Ireland

### DSP

In general, from Jan–mid June, all samples were negative. Levels were observed to increase in Bantry Bay Outer (Castletownbere) from mid June, with the first positive occurring in this area at the end of June. This coincided with the increasing numbers of *Dinophysis* sp. observed in all areas within Bantry Bay, with the highest number recorded in Castletownbere (320 cells L<sup>-1</sup>) during June. The toxicity was observed to increase further in all of the production sites in the Inner part of Bantry Bay above the regulatory limit from early July, and continued to increase further throughout August, where levels were observed to peak during the last week of August, where the highest level observed was 1.84 µg g<sup>-1</sup> OA eq. total tissue (Glengarriff, Bantry Inner).

Levels of *Dinophysis* sp. continued to increase throughout July–August, where levels peaked in all areas along the south and southwest coasts in August. Highest levels observed were in Bantry (9320 cells L<sup>-1</sup>). Also from the beginning of August, toxicity above the regulatory limit was observed for OA equivalents in the Kenmare Bay region, predominantly in two areas, Ardgroom and Kilmakilloge, where the highest OA levels were observed nationally in these areas, 2.22 and 3.83 µg g<sup>-1</sup> OA eq., respectively, during the last week of August. Further closures in the South West during August included Dunmanus and Roaringwater Bays where the highest OA levels observed were 0.82 and 0.32 OA eq., respectively.

Numbers of *Dinophysis* sp. were observed to further decrease during the months of September and October and were observed in the South West at low levels, typically 40–280 cells L<sup>-1</sup>. The levels of OA equivalents were also observed to decrease further in samples of *M. edulis* submitted from the above areas. Due to decreasing levels of OA equivalents and Management Cell Decisions, a number of areas in the southwest were opened towards the end of October including sites in Bantry Inner and Outer.

During November levels of OA equivalents were observed to persist in a number of sites within the Bantry and Kenmare areas around the regulatory limit during the shoulder toxicity period. Levels of OA equivalents were observed to decrease further below the regulatory level during December, with all areas placed on Open status.

In August, samples of the queen scallop, *A. opercularis*, were submitted from Middle Ground Donegal, OS-MG-MG (39-E3) and were observed to contain levels of OA equivalents above the regulatory limit, maximum level observed 1.06 µg g<sup>-1</sup> OA eq. total tissue. The OA present was almost entirely in the form of okadaic esters.

### PSP

There was no recorded AZP or PSP toxicity above the regulatory level observed in samples from the South West. In the south however, PSP toxins were detected 22/06/04 in samples of *M. edulis* in Cork Harbour (levels of ca. 65 µg STX eq. 100 g<sup>-1</sup>) in June. The highest number of cells observed was 75,800 cells L<sup>-1</sup> in Cork Harbour, North Channel (15/06/04). This level

was observed to decrease the following week to  $45 \mu\text{g } 100 \text{ g}^{-1}$  STX eq. whole flesh, and was below the limit of detection for the AOAC bioassay the following week.

High numbers of *Alexandrium* sp. were also observed in June, in Oysterhaven  $23,280 \text{ cells L}^{-1}$  and also in Wexford Harbour  $>8,360 \text{ cells L}^{-1}$ . Samples of *C. gigas* and *M. edulis* from these respective areas were observed to be positive using the Jellett PSP Rapid Test Kit (screening method) but negative under AOAC PSP bioassay (confirmatory technique). This was due to the lower level of sensitivity observed of the test kits (lower LOD (ca.  $25\text{--}40 \mu\text{g STX eq. } 100 \text{ g}^{-1}$  – observed from naturally contaminated PSP material) than the AOAC PSP bioassay (ca.  $>40 \mu\text{g STX eq. } 100 \text{ g}^{-1}$ )).

### ASP

Highest levels of domoic and epi-domoic acid in the gonad tissues of *Pecten maximus* in off-shore areas were observed in Wexford Ground (VIIa ICES Rectangles 33-E3 (March) and 33-E4 (October)) with maximum levels observed of  $29.8 \mu\text{g g}^{-1}$  domoic acid. In inshore areas, the highest levels observed in gonad tissues were in Crookhaven in February ( $103.68 \mu\text{g g}^{-1}$  domoic acid) and levels were also observed to be above the regulatory limit in Bantry and Valentia Harbour. Whole flesh samples of *M. edulis*, *C. gigas*, *O. edulis* and *T. philipinarum* analysed for the presence of domoic and epi-domoic acid were all observed to be below the regulatory limit of  $20 \mu\text{g g}^{-1}$  domoic acid).

## 9.8 Latvia

The HAB situation in the Gulf of Riga (Eastern Baltic Sea) in 2004 is reported from the Latvian National Monitoring Program. HAB studies involved the identification of HAB species, detection of cell densities and biomass. No analyses for algal toxins were performed. Sampling sites covered the whole Gulf with stations in the coastal zone visited more frequently.

The number of HAB species in the Gulf of Riga decreased from the 10 species observed in 2003 to 8 species in 2004. *Aphanizomenon flos-aquae*, *Anabaena spiroides*, *Nodularia spumigena*, *Dinophysis acuminata*, *Dinophysis rotundata*, *Prorocentrum minimum*, *Chaetoceros danicus* and *Chrysochromulina* spp. were observed. The nitrogen fixing cyanobacteria - *Aphanizomenon flos-aqua* dominated. Other organisms observed included: dinoflagellates - *Dinophysis acuminata*, diatoms - *Chaetoceros danicus*, and cryptophytes - *Chrysochromulina* spp.

*Aphanizomenon flos-aquae* was detected throughout the sampling period (January–December) and when it was at its highest concentration ( $334.7 \text{ mg m}^{-3}$ ), it was more than 50 % the total phytoplankton biomass (1.6 times higher than in 2003).

The maximum concentrations of *Nodularia spumigena* were observed at the end of August ( $19.4 \text{ mg m}^{-3}$  in offshore). In comparison with 2003 the biomass was 1.7 times lower and was only 4 % of the total phytoplankton biomass.

Development of *Anabaena spiroides* was episodic with the maximum ( $2 \text{ mg m}^{-3}$ ) in the coastal zone, in regions with relatively high nutrient concentrations.

*Dinophysis acuminata* was observed throughout the year – reaching its maximal biomass offshore ( $61.7 \text{ mg m}^{-3}$ ). *D. acuminata* composed 10% of the total phytoplankton biomass and was 1.2 times higher than in 2003.

HABs did not result in any harmful effects during 2004.

## 9.9 The Netherlands

Several potentially harmful and toxic phytoplankton species were observed in 2004: *Phaeocystis globosa*, *Noctiluca scintillans*, *Pseudo-nitzschia* spp., *Myrionecta rubra*, *Alexandrium tamarense* and *Dinophysis acuminata*. No harmful effects were observed and there were no reports of toxicity in bivalves.

### Wadden Sea and Delta area:

Species of the potentially toxic diatoms *Pseudo-nitzschia*, e.g., *P. delicatissima*, *P. pseudodelicatissima* and the *P. pungens-multiseriis* complex, were regularly observed at background levels. Cell counts ranged from 0–50,000 cells L<sup>-1</sup> over the period May through September, with a peak in June. These numbers were observed in the Wadden Sea and Oosterschelde (Delta area). The presence of *Pseudo-nitzschia* species did not result in any toxic events.

*Dinophysis acuminata* was observed in Lake Grevelingen (Delta area) in August at cell counts greater than 160 cells L<sup>-1</sup>. *D. acuminata* has also been reported in background levels (0–50 cells L<sup>-1</sup>) in several areas, such as the Wadden Sea and Oosterschelde in August. There were no reports of toxicity of bivalve mollusks in Dutch coastal waters.

### North Sea (coast)

The annual *Phaeocystis globos* bloom started in the second half of April, reached 40 million cells L<sup>-1</sup>, and declined in the second week of May. No harmful effects were reported. *P. globosa* colonies were observed until August.

*Noctiluca scintillans* was counted in numbers ranging from 100–500 cells L<sup>-1</sup> from the end of May until the beginning of August. In this period *Myrionecta rubra* was observed occasionally. *Pseudo-nitzschia* spp. were observed from April until August.

*Alexandrium tamarense* was found in July and August in low concentrations ( $\leq 100$  cells L<sup>-1</sup>). It is still unclear if these ‘coastal’ *Alexandrium*’s are toxic: the Dutch coastal waters originate from the English Channel (home of the non-toxic *A. tamarense* clade). In 2001 *A. tamarense* was isolated from a sample taken 135 km off-shore, in a region influenced by North-Eastern English coastal water (home of a toxic *A. tamarense* clade). This isolate was cultured and its toxicity was established using HPLC analysis.

*Dinophysis acuminata* concentrations, in July and August, were equally low ( $\leq 100$  cells L<sup>-1</sup>). In summary, potentially toxic phytoplankton were observed in low concentrations, and no harmful effects were observed.

## 9.10 Norway

### ASP

Domoic acid (ASP toxin) was not detected at levels above quarantine levels in mussels along the Norwegian coast during 2004.

### DSP

As usual, DSP-toxins were recorded above quarantine levels at some monitoring stations in southern Norway. The problem in the south was, however, less than average, and occurred mainly from October and onwards. In northern Norway, on the other hand, DSP toxin levels in 2004 were the highest since regular monitoring was established, about 10 years ago. Already from early in August *Dinophysis acuta* was common in the area and caused accumulations of DSP toxins above quarantine levels.

## PSP

Occurrences of PSP-toxins in mussels are recurrent problems in Norway. In 2004, these problems were relatively small, and occurred at a few stations along the west-coast in March–June and at some stations in northern Norway in April–June.

## AZA (Azaspiracides)

For the second time in Norway, the presence of azaspiracides in mussels exceeded quarantine levels. It happened at a couple of the monitoring stations on the west coast in October. We are not sure as to the potential source organism(s).

### 9.11 Poland

In the Gulf of Gdansk a small number of toxic *Nodularia* filaments were observed for the first time at the beginning of June when water temperature reached over 16°C. Then the toxic cyanobacteria disappeared for nearly a month, which was probably the result of water temperature decrease and water mixing by a strong wind.

In coastal waters of the Polish zone of the Baltic Sea, a detectable level of nodularin toxin (1.2 µg L<sup>-1</sup>) was recorded for the first time on 19 July. With the increase in surface water temperature, *Nodularia* became a prevailing phytoplankton component. In coastal waters *Nodularia* reached maximum cell numbers of 23.6 million cells L<sup>-1</sup> at the turn of July and August. At this time the maximal cell-bound nodularin concentration was 25.8 mg L<sup>-1</sup> and in lyophilized phytoplankton samples, 3.8 mg g<sup>-1</sup> dry weight. The toxin was detected in blue-mussels, fish and sediments. During the high biomass period of the toxic cyanobacteria, fish kills were observed. Scum accumulated along the shore and caused occasional beach closers. Increased numbers of people complaining about skin irritation and allergic reactions were recorded in 2004. Even when the massive bloom of toxic *Nodularia* did not last longer than 10 days, nodularin could still be detected in phytoplankton samples in early September.

This year, at least three morphospecies of *Nodularia* were recorded: straight, waved and coiled. The most abundant was the coiled form with vegetative cells slightly smaller than in other forms.

There were no records of *Alexandrium* blooms in Polish coastal waters in 2004.

### 9.12 Portugal

In Portugal, as in 2003, no PSP toxins were detected.

DSP toxins associated with *Dinophysis acuminata* and *D. acuta* were observed on the north-west coast, mainly in the Aveiro area (from June to November), and on the Algarve coast (from July to September) and caused harvesting closures of *Mytilus edulis* and *Donax* sp. respectively.

ASP toxins caused by *Pseudo-nitzschia* spp., were recorded above the regulatory levels in the Tagus estuary (April) and Aveiro (May), both sites were closed for carpet shell and other molluscan bivalve harvesting.

Domoic acid levels >20 µg g<sup>-1</sup> were detected in scallops from one site on the northwest coast during 2004.

### 9.13 Scotland

Low numbers of *Alexandrium* spp. cells were observed around the Scottish coast during 2004. The maximum number of cells observed is 1,900 cells. L<sup>-1</sup> in Shetland. Levels of PSP greater than the closure level of 80 µg STX eq. 100g<sup>-1</sup> were not observed in *M. edulis*.

*Dinophysis* cell numbers were less than those observed in previous years with a maximum cell density of 1,900 cells L<sup>-1</sup> observed at Southannan. *Dinophysis acuminata* dominated the population during 2004. *D. acuta* levels were much reduced.

*Pseudo-nitzschia* spp. again occurred in high numbers throughout 2004. Blooms of *Pseudo-nitzschia delicatissima* type were observed during the March/April 2004 while *Pseudo-nitzschia seriata* type species were observed in August and September. Closures of the off-shore scallop fishing grounds for concentrations of domoic acid greater than the closure limit were again enforced throughout the year.

## 9.14 Spain

Toxic events during 2004 fall into the category of normal chronic harmful events, with the exception of the Cantabrian region, where DSP toxicity associated with populations of *Dinophysis acuminata* were reported for the first time. Monitoring of potentially harmful phytoplankton and of toxins in shellfish is a responsibility of the Autonomous Governments. The Galician Monitoring Centre performed analyses for the Cantabrian Government, and the Catalonians have carried out periodic sampling in the Balearic Islands during the last years.

### Andalucia

Diarrhetic Shellfish Poisoning toxins associated to proliferations of *Dinophysis acuminata* (max. concentration, 3737 cell L<sup>-1</sup>) caused closures of shellfish exploitations, specially those of Donax clams (*Donax trunculus*) in the Atlantic coast of Andalucia (Gulf of Cádiz), between January and July.

On the Mediterranean coast (Alboran Sea), PSP toxins produced by *Gymnodinium catenatum* (max. level, 2812 cell L<sup>-1</sup>) led to harvesting closures of warty Venus (*Acanthocardia tuberculata*), scallops (*Pecten maximus*) and mussels (*Mytilus galloprovincialis*) from January to mid-March. A new outbreak in August (max. level, 3440 cell L<sup>-1</sup>) affected practically all commercially exploited shellfish resources. Maximum toxin content during both events were observed in warty Venus and mussels.

### Catalonia and Balearic Islands

As usual, a high diversity of harmful algae events in very localized areas were observed during 2004. *Alexandrium taylorii* caused water discolorations that upset the tourist industry in La Fosca Beach (Gerona), and the islands of Mallorca and Ibiza (Balears).

The PSP producer, *Alexandrium minutum* appeared widespread throughout the whole Catalan coast between February and August. Maximum concentrations were found in Arenys (>2x10<sup>6</sup> cell L<sup>-1</sup>) in the end of March. PSP toxin levels were below quarantine levels in Delta del Ebro.

*Dinophysis sacculus* (DSP) reached high numbers (up to 15,600 cell L<sup>-1</sup>) in February–March, June and August in different spots of the Catalan coast but DSP toxins did not reach quarantine levels in the aquaculture sites at the Delta del Ebro.

High concentrations (up to 10<sup>5</sup> cells L<sup>-1</sup>) of the benthic dinoflagellate *Ostreopsis siamensis* were observed to be resuspended in the water column in touristy beaches in August. There were 40 reports of human respiratory irritation at the beach of Llavaneras.

### Galicia and Cantabrian Sea:

**DSP outbreaks:** 2004 was a very bad year for DSP outbreaks. Proliferations of *Dinophysis acuta* until the end of 2003 caused closures of mussel harvesting in the Rías Baixas (Muros, Arousa, Pontevedra and Vigo) that lasted until mid-February of 2004. Intermittent closures associated with proliferations of *Dinophysis acuminata* affected the whole Galician coast from

the end of April to early October. New proliferations of *D. acuta* caused new closures in the Rias Baixas from early October until the end of the year. Some high risk mussel growing areas in Ria de Pontevedra were closed to harvesting for up to nine months. Concentrations of *Dinophysis* causing this damage never exceeded  $3 \times 10^3$  cells  $L^{-1}$  in integrated (hose sampler) water samples. For the first time, DSP toxins associated with *Dinophysis acuminata* were reported in shellfish from the Cantabrian coast.

**PSP outbreaks:** Very localized dense blooms of *Alexandrium minutum* (up to  $4 \times 10^5$  cells  $L^{-1}$ ) developed between May and July in the Bay of Baiona (Ria de Vigo) and in Ria de Ares (Rias Altas) leading to minor short-lasting closures of mussel harvesting. New blooms of the same species occurred in Baiona Bay in September.

**ASP outbreaks:** Proliferations of domoic acid-producers species of *Pseudo-nitzschia* caused minor closures of mussel harvesting (1–2 weeks) in all the Galician Rías between March and early June, and from September to mid-October. Nevertheless, the effects of *Pseudo-nitzschia* blooms have been devastating for the scallop (*Pecten maximus*) growers. Pectinids show a high affinity towards domoic acid and are hardly able to detoxify themselves between consecutive episodes. Scallops were harvested only from restricted areas of Ria de Arousa.

## 9.15 Sweden

### (Skagerrak and Kattegat) - Harmful algae

A bloom of the raphidophyte *Chattonella* sp. occurred in the area in March with cells densities of up to 730,000 cells  $L^{-1}$  along the coast of Halland. No harmful effects were observed but this species is to affect fish gills. An intense bloom of the coccolithophorid, *Emiliania huxleyi*, was observed in the end of May and beginning of June. This non-harmful algal bloom caused a lot of interest from the media due the water discoloration. The diatom *Pseudo-nitzschia* sp. (*delicatissima*-group) was observed in cell densities up to 2.3 million cells  $L^{-1}$  in June in the Havstensfjord. No harmful effects were observed. Dinoflagellates producing diarrhetic shellfish toxins (DST) were found in the area all year. *Dinophysis acuminata* and *D. acuta* were observed at abundances above the warning limits (limits are 900 cells  $L^{-1}$  and 200 cells  $L^{-1}$  respectively). In May and June the paralytic shellfish toxin (PST) producing dinoflagellate *Alexandrium* spp. was observed in abundances above the warning limit (200 cells  $L^{-1}$ ). The highest abundance of *Alexandrium* sp. was 1780 cells  $L^{-1}$  in the beginning of May at station Havstensfjord. *Lingulodinium polyedrum*, a producer of yessotoxins, cells were observed in July, August and September with cell densities of up to 20,000 cells  $L^{-1}$ . Yessotoxins were detected in blue mussels.

The potentially harmful algae *Heterosigma* sp., *Dictyocha speculum*, *Chrysochromulina* spp., and *Pseudo-nitzschia* spp. occurred in the area but in moderate abundances.

### (Skagerrak and Kattegat) Algal toxins in blue mussels (*Mytilus edulis*)

From January to March and from the end of September to the end of the year concentrations of DST (diarrhetic shellfish toxins) were above the limit for marketing in some areas of the Swedish Skagerrak coast. In 2004, the concentrations of DST were low compared to the average values measured from 1988–2004. It should be pointed out that in other areas, mussels with DST content below the quarantine limit were harvested simultaneously, thus safe mussels were available through most of year 2004. Paralytic shellfish toxins (PST) above the quarantine limit were not detected during 2004.

### **Baltic Proper**

An observation of the potentially toxic cyanobacteria *Aphanizomenon* sp. was made as early as April between the islands Öland and Gotland. Substantial blooms of large cyanobacteria were observed in large parts of the Baltic proper from ca 7 July to the end of August. Substantial surface accumulations were observed at the end of July and especially ca 6–10 August. Tourism was affected since swimmers were advised not to go into the water in some areas. Also the public found the cyanobacteria to be a nuisance.

### **Bothnian Bay**

In the beginning of August a strong bloom of the cyanobacteria *Nodularia spumigena* was observed along part of the Swedish coast. The organism was found to be toxic and warnings were issued. This is the first observation of this type of bloom in the area. Also an off shore bloom was observed using satellite imagery in August. Cyanobacteria are probably the organisms observed but no samples were obtained for microscopy.

## **9.16 USA**

2004 was basically a “normal” year for HABs in the U.S., with several noteworthy or exceptional events.

### **PSP**

Similar to previous years, Maine, California, Oregon, Washington, and Alaska all recorded PSP events during 2004. Both eastern and western Maine experienced closures due to high toxin levels (maximum of 41,369 µg STX eq. 100 g<sup>-1</sup>). This occurred as a result of a late-season bloom that started in 2003 and continued into 2004. The southern coast of Oregon saw elevated PSP toxin levels in late summer and fall and closed this area to recreational shellfish harvesting.

During 2002, the first Puffer Fish Poisoning (PFP) attributed to saxitoxin was reported in the U.S. (Florida – east coast). Since that time, the state has had a ban on the harvest of puffer fish from the India River Lagoon area and routine PFP monitoring was implemented at that time. In 2004, puffer fish in this area were once again found to contain saxitoxin, presumably taken up by feeding upon small infaunal bivalves that were also found to contain saxitoxin. The presumed toxic organism is *Pyrodinium bahamense*. There were 5 human cases of PFP in 2004.

### **ASP**

ASP was recorded in California, Oregon, and Washington. Similar to the last two years, the outbreak in California was extensive, stretching from Los Angeles to northern California, but the toxin levels were not very high – with 170 ppm domoic acid being the highest number reported.

### **NSP**

*Karenia brevis* blooms occurred in southwest and northwest Florida. Fish kills and human respiratory irritation were reported along with the Florida blooms. Between March 10 and April 13, 2004, 107 bottlenose dolphins were stranded dead along the Florida Panhandle. Hundreds of dead fish and marine invertebrates were also discovered in the area. This event is attributed to *Karenia brevis*.



### **Brown tide**

For the second time since 1985, there were no reports of brown tide this year in New Jersey or Long Island. In Maryland, however, *Aureococcus anophagefferens* cell concentrations reached Category 3 levels (as per Gastrich and Wazniak 2002 Brown Tide Bloom Index,  $\geq 200,000$  cells  $\text{mL}^{-1}$ ) in spring 2004 in Maryland's Coastal Bays. Recent studies in the coastal bays have shown brown tide abundances as low as 20,000 cells  $\text{mL}^{-1}$  may affect juvenile hard clam (growth and survival).

### **Pfiesteria**

There were no reports of fish kills definitively attributed to *Pfiesteria* in North Carolina or Chesapeake Bay.

### ***Prymnesium parvum* "Golden Algae"**

During 2004, Texas experienced toxic *Prymnesium parvum* blooms in freshwater reservoirs and rivers, which caused extensive fish kills – both natural and aquacultured fish.

During 2004, the East Coast of Florida experienced *Prymnesium parvum* blooms causing mass mortalities of fish and birds.

### **Cyanobacteria**

Cyanobacterial blooms occurred in May in the upper Chesapeake Bay. There were anecdotal reports of a waterman experiencing flu like symptoms, and a dog experiencing seizures after spending time on and in bloom waters. Microcystins were detected in all samples collected. Beach closures were enacted on two tributaries during the bloom period. Cyanobacterial blooms dominated the surface algae for the summer of 2004 on the Potomac River. Concentrations up to 80 million cells  $\text{mL}^{-1}$  were detected. Microcystins were detected in all samples tested during the summer. Anatoxins in very low concentrations were detected late in summer (confirmed by two labs) when the bloom had co-dominants of *Anabaena* and *Microcystis*. An anecdotal report was received of a family that swam in Virginia waters subsequently developing severe rashes. Recreational fishermen in the region reported extremely low fishing success throughout the bloom period.

### **Macroalgae**

A severe macroalgae bloom was observed in the upper Chesapeake Bay in 2004. Starting in May, summer conditions coincided with the best water clarity observed in the region in the 20 years history of the Bay monitoring program. Nevertheless, the macroalgal bloom covered roughly 20 miles and basically shut down the crabbing and netting efforts of fishermen in that area for the late spring and summer months. The bloom was a mix of macroalgal species involved including *Lyngbya latissima*, *Cladophora* sp. and *Rhizoclonium* sp. In some areas, the submerged aquatic vegetation was overgrown by the macroalgae in mats. There were also reports of abundant *Ulva* in tributaries farther south in the Bay.

### **Unusual Events**

*Takayama pulchella* was responsible for over 10,000 dead fish (snook, snapper and mullet) in a Florida east coast lagoon. The organisms were to blame for fish kills in the same area of the lagoon in 1990 and 1996, the only other times the algae have been identified in North America,

## 10 Term of Reference G

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Review progress in computerized production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years

Monica Lion reported on the progress in computerized production of decadal maps from the HAE-DAT database.

The new MySQL/PHP platform of HAE-DAT allows for the computerized production of different worksheets that have been tested in a pilot study using information from the USA, Portugal and Spain. These worksheets were sent to Catherine Belin (IFREMER), to check their compatibility with the production of new decadal maps. These new map products are made with the softwares ArcView and Flash.

At this initial trial, searches were focused on “per year effects” in any HAE-area, and other parameters such as “toxins per year” and “species distribution” were extracted. Examples of the three types of maps created by IFREMER were presented.

Until recently, the decadal maps only showed the presence of toxins or mortality observations from the last 10-year period. When HAE-DAT is fully linked with the maps, it will be possible to generate maps for any particular period or specific year. These maps can include: shell-fish syndromes, phytoplankton species or any other HAE-DAT parameter.

The linkage between HAE-DAT and the GIS maps is in process for ICES member countries and it can be extended to countries outside ICES as well as other types of maps. In the near future, this will allow a nearly automatic generation of maps.

The WGHABD considered the potential for the production of annual and species distribution maps to be very useful.

## 11 Term of Reference H

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Propose types of analyses that should be performed using the IOC-ICES HAE-DAT dataset and identify problems and gaps in this dataset that must be rectified before the analyses can be conducted

Monica Lion reported on the update and progress of the transformation of the present HAE-DAT (Access 97 software) into a more user friendly and manageable format.

HAE-DAT presently is current until 2004. All 2003 national reports have been entered manually into the database - which now has a total of 1626 reports.

During this past year, an effort was made by the IOC-IEO SCCCHA, with collaboration from Benjamin Sims from the IOC Headquarters, to establish a new electronic format. Before this update, HAE-DAT had to be downloaded from the Internet and ran under the Access<sup>®</sup>97 programme. HAE-DAT is now being transferred from the Microsoft Access<sup>®</sup>97 desktop solution into a MySQL/PHP platform which runs on a Linux server which allows for computerized production of different worksheets.

During the data transfer process to the new MySQL format, some issues identified in the 2004 WG report that required editing by designated country delegates should have been solved by the data managers in order to homogenize the tables and fields required. This caused delays in the new format completion.

The most important modifications were introduced in the following:

- Country codes, which have been edited following the ISO short country names (ISO 3166-1-alpha-2 code elements);

- Information on precise event location - required the conversion of different formats into degrees, minutes and seconds.
- Introduction of the new HAE areas which replace current ICES areas.
- Causative and additional species information - which required the edition of the numerical format of the cellular count, the creation of taxonomical classes and causative species lists.
- Environmental conditions which required the homogenization of the numerical format of different fields.
- Harmful effects which required the creation of drop down menus for parameters such as toxin and assay type information.

Due to the fact that there is not a complete updated reference list that has all the necessary information compiled (such as causative and additional species lists, as well as the taxonomical classes), lists have been created and required additional efforts. The 'IOC Taxonomic Reference List of Toxic Algae', Moestrup, Ø. (Ed.): Intergovernmental Oceanographic Commission of UNESCO; [ioc.unesco.org/hab/data.htm](http://ioc.unesco.org/hab/data.htm), 2004, was used to create the toxic species list and 'Identifying Marine Phytoplankton', Tomas, C. (Ed.), Academic Press, 1997, was used to create the additional species list, in addition to personal communications with members of the Task Team on Algal Taxonomy (IOC-HAB).

As soon as the new format is operational, country delegates will check the accuracy of the records that have been edited by the data managers and will comment, complete or edit any inconsistencies.

With the goal of HAE-DAT becoming the global database on harmful algal events, during this past year some non-ICES countries agreed to join and submit records of events on the systematic and annual submission forms for harmful algal event reporting to HAE-DAT. This has been the case with the IOC-HANA (Harmful Algae in North Africa) group and some other Mediterranean countries such as Italy and Greece. In October 2004, PICES (Pacific International Council for the Exploration of the Sea) formally joined the group reporting to HAE-DAT and tested the reporting data format during their PICES/IOC Workshop on "Harmful Algal Blooms-Harmonization of data" in Honolulu, Hawaii, USA.

In the future, the plan is for HAE-DAT to continue to grow by adding new records and more countries. Additional parameters will include the integration of additional data related to describe events from monitoring programmes such as MON-DAT, etc.

The WGHABD proposed that a subset of "a verified dataset" from HAE-DAT be submitted to the WGSEAM to find out which types of statistical analyses would be suitable for the dataset and to identify appropriate tools for sporadic event analysis within HAE-DAT.

It was stressed the HAE-DAT is a Meta-database, and the analysis of information from it should be done bearing this in mind; for instance, in some Norwegian sample sites there may be limited environmental data to coincide with the phytoplankton data.

## 12 Joint Theme Session ASC 2006

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Discuss the joint WGHABD and WGPBI theme session in 2006 at ICES ASC "Harmful Algae Bloom Dynamics; Validation of model predictions (possibilities and limitations) and status on coupled physical-biological process knowledge"

It was suggested that this was a useful approach to incorporate numerical tools into the work of the WGHABD. A co-chair from the WGPBI from Finland Tipani Stipa has been appointed. It was proposed to contact Patrick Gentien to ask them to act in this role. Patrick Gentien (France) accepted the request to act as WGHABD co-chair.

## 13 Proposed Terms of Reference 2005

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics [WGHABD] (Chair J. Silke, Ireland) will meet in Gdynia, Poland, from 3–6 April 2006 to:

- a. Review progress in the detection of harmful algal blooms and their dynamics by remote sensing techniques and examining results from new sensors and algorithms as well as validation procedures used for HAB observations.
- b. Review the section on Phytoplankton Monitoring in the Report of the Joint FAO/IOC/WHO Ad Hoc Expert Consultation for *Codex Alimentarius* on Biotoxins in Bivalve Mollusks (Oslo 26 September 2004).
- c. Review the outcome of the WKNCT Workshop on New and Classical Techniques in Enumeration of Phytoplankton.
- d. Review progress towards the joint theme session between WGHABD and WGPBI for the ICES ASC in 2006 titled “Harmful Algae Bloom Dynamics; Validation of model predictions (possibilities and limitations) and status on coupled physical-biological process knowledge”.
- e. Review progress and analyses that REGNS North Sea Group have done on datasets submitted by members of WGHABD (to meet in the interim).
- f. Discuss new findings that pertain to harmful algal bloom dynamics. Bring new findings in phytoplankton population dynamics models, with emphasis on loss processes, to the attention of WGHABD for discussion.
- g. Review the on-line format of HAEDAT submission form and evaluate the amendments made to update historical submissions and links to mapping.
- h. Review the structure and composition of the decadal HAE maps for the ICES region with special reference to clarifying the distinction between harmful algal blooms and the harmful affects that are reported on the maps. In particular, the registration of cyanobacterial blooms in brackish and marine waters should be revisited from the emerging perspective of their known toxicity and implicit harmful effects.
- i. Collate and assess National reports and update the decadal mapping of harmful algal events for the IOC/ICES harmful algal database, HAE-DAT (Country Reps).

### Supporting Information

Priority:	The activities of this group are fundamental to the work of the Oceanography Committee. The work is essential to the development and understanding of the effects of climate and man-induced variability and change in relation to the health of the ecosystem. The work of this ICES-/IOC WG is deemed high priority.
Scientific Justification and relation to Action Plan:	<p>Action Plan No: 1.1, 1.2, 1.5, 1.7, 1.10, 1.11, 1.12, 2.3, 2.9, 3.2, 4.11, 5.10, 5.13, 5.16, 6.1, 6.2, 6.3, 6.4, 8.1, 8.2, 8.4.</p> <p><b>Term of Reference a)</b> Space – or airborne remote sensing of the sea, sometimes termed EO (Earth Observations) is often motivated with the aim of observing harmful algal blooms. Initiatives including the GMES (Global Monitoring for the Environment and Safety), the MERSEA program and its application to Operational Oceanography and HAB detection in real time will be reviewed. New satellites and sensors have become operational the last years, i.e., the MERIS sensor on the European satellite ENVISAT and the US satellites AQUA and Terra with the sensor MODIS. Older sensors include the SeaWiFS that has reached its end of life. Earth observations (EO) have limitations regarding HAB observations which include that only high biomass blooms are detected, only surface water is monitored etc. In general the only HAB-product available is chlorophyll <i>a</i>. Also cloud cover is a problem for the technique. New sensors with higher spatial and spectral resolution as well as new algorithms for data processing hold promise for resolving signals for HAB-species or algal groups, e.g., cyanobacteria. There is a great need to review the results from the new sensors and algorithms and the validations procedures used.</p> <p><b>Term of Reference b)</b> See how the recommendations of the expert consultation fit the ICES countries’ governmental strategies on biotoxin management and linkages to HAB dynamics.</p>

	<p><b>Term of Reference c)</b> This workshop, a complex activity requiring algal cultures, field material and a variety of different methodologies, will provide valuable results on the application of different microscope based techniques and some advanced molecular techniques for the identification and quantification of harmful microalgae. The WGHABD was instrumental in initiating this process and established a steering committee. It is appropriate that the WG evaluates the report from the workshop and promotes its dissemination.</p> <p><b>Term of Reference d)</b> In spite of large gaps of basic process knowledge around HAB dynamics, several 3D modelling initiatives are ongoing with respect to studying and predicting HABs. It is due time to couple the expertise of modellers and biologists to reveal the most urgent needs for better process knowledge to improve the predictability of models. The session aims at participation from 3D modellers and biologists interested in explaining the occurrence, initiation, development and decay of HABs in time and space.</p> <p><b>Term of Reference e)</b> The REGNS study group has requested that the WGHABD prepare to provide data, information and indicators. A delegate from the WGHABD will attend the REGNS meeting in May 2005 and will report to the group in 2006 on the progress of the assembly and analysis of the data.</p> <p><b>Term of Reference f)</b> Modelling exercises aimed at understanding HAB population dynamics have suffered from poor estimates of biological loss terms. Current knowledge on selected HAB loss processes (e.g., grazing, viruses, parasitism, and programmed cell mortality) is limited. Improved knowledge on the dynamics of these loss processes and their relative contribution is essential to improve models for HAB dynamics.</p> <p><b>Term of Reference g)</b> The HAEDAT database is currently being updated to incorporate a more user friendly and consistent format both in the structure of the database and the report submission. It is proposed to demonstrate the new additions to the system. A number of amendments to historical data are currently being made by the data administrator and these will need evaluation by the national representatives. It is an objective of the IOC to link the HAEDAT with a mapping capability, and progress in this area will be demonstrated and reviewed.</p> <p>The ability to perform statistical analysis of the data is an important function of this database to allow for the full potential of this resource to be realized. Links with the ICES Working Group on Database Management will be initiated to get suggestions on the most appropriate analyses.</p> <p><b>Term of Reference h)</b> The criteria for reporting of HAE of the well-known toxin syndromes (PSP, DSP, NSP, ASP, etc.) on the decadal maps are currently inconsistent with those for cyanobacterial blooms, particularly in the Baltic region. The WG should consider making amendments to the reporting procedures to rectify these discrepancies. In some cases, the titles on the maps (e.g., "presence of toxins") also do not accurately reflect the information reported</p> <p><b>Term of Reference i)</b> The work of collating the national HAE reports and building up HAE-DAT and the associated maps is an activity which is unique to the WGHABD. HAE-DAT is not yet established enough to stand alone. A critical step forward is to make HAE-DAT operational with input from regions/countries outside the ICES areas as originally envisaged. PICES, South America, HANA and Caribbean countries (via IOC/FANSA and IOC/ANCA) are now included in HAE-DAT. It should be endeavoured to include HAE-DAT and the associated decadal maps as a contribution to GOOS, thereby embedding these activities in a permanent setting and securing continuity.</p>
Resource Requirements:	The research programmes which provide the main input to this group are already underway, and resources already committed. The additional resource required to undertake additional activities in the framework of this group is negligible.
Participants:	The Group is normally attended by some 20–25 members and guests
Secretariat Facilities:	None
Financial:	No financial implications
Linkages To Advisory Committees:	There are no obvious direct linkages with the advisory committees
Linkages To other Committees or Groups:	WGHABD interacts with WGZE, WGPE, SGGIB, SGBOSV, WGPBI.

Linkages to other Organisations:	The work of this group is undertaken in close collaboration with the IOC HAB Programme. IOC should be consulted regarding ToR or discontinuation of the WG prior to the ASC. There is a linkage to SCOR through the interactions of the IOC-SCOR GEOHAB Programme.
Secretariat Marginal Cost Share:	ICES

## 14 Recommendations

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WGHABD recommends that officers of Iceland and the Faroe Islands identify national focal points/ individuals responsible for data submission to HAE-DAT, decadal maps and national reports. It should be emphasised that these reports should be submitted even if the delegates is unable to attend the meeting. It is recommended that these focal points are identified by December 2005.

It was proposed that Henrik Enevoldsen contact ISSHA to establish what their objectives are regarding event and distribution information from published sources.

The WG requests that the ICES Data management group be contacted to evaluate the potential for statistical analyses from the HAEDAT database.

WGHABD requests that the MONDAT questionnaire be circulated to all participating countries to establish current action levels of toxic phytoplankton in various geographical locations.

## Annex 1: List of participants

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## Annex 2: Agenda

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### Monday, 4 April 2005

- 09:00 Welcome; housekeeping issues
- Introduction of participants
- Review by the Oceanographic Committee
- Adoption of agenda/ Terms of Reference
- 09:30 **ToR a**: review the dynamics of toxin producing phytoplankton and associated toxins in shellfish, related to phytoplankton abundance, and phytoplankton community structure with references to HAB population dynamics. In 2005 the focus will be toxin producing phytoplankton and associated toxins in shellfish (Canada, Spain, Scotland, US, Denmark) (Einar Dahl, Beatriz Reguera, Jennifer Martin, others.....)
- 10:30 ***Health break***
- 11:00 ToR a continued.....
- 11:30 **Tor h**: propose types of analyses that should be performed using the IOC-ICES HAE-DAT dataset and identify problems and gaps in this dataset that must be rectified before the analyses can be conducted (Monica Lion, others.)
- 12:30 ***LUNCH***
- 13:30 **ToR g**: review progress in computerized production of decadal maps from country reports, including the revision of reports already in the database covering the last 10 years (Monica Lion, others)
- 14:45 **Modelling of Harmful Algae Blooms** (Morten Skogen) (ToR c)
- 15:30 ***Health Break***
- 16:00 **ToR f**: Collate and assess National reports and update the decadal mapping of harmful algal events for the IOC/ICES harmful algal database, HAE-DAT (Country Reps)
- 18:30 Adjourn for the day

### Tuesday, 5 April

- 08:30 **ToR d**: Review plans for the proposed Workshop on New and Classic Techniques for the determination of numerical abundance and bio-volume of HAB-species (Bengt Karlson, .....)
- 09:30 Discuss joint WGHABD and WGPBI theme session in 2006 at ICES ASC “Harmful Algae Bloom Dynamics; Validation of model predictions (possibilities and limitations) and status on coupled physical-biological process knowledge” **Appoint co-chair**; general discussion
- 10:30 ***Health break***
- 11:00 **ToR e**: prepare data on the distribution and number of harmful algal blooms in the North Sea for the period 1984-2004,(where available), and submit the data to the secure REGNS website in excel spreadsheet format in preparation for the REGNS Integrated Assessment Workshop to be held from 9-11

May 2005. The data should be averaged and presented in ICES grid spatial scale (Allan Cembella, Lars Naustvall, others .....)

#### General Discussion

- 12:30 **LUNCH**
- 13:30 Report writing; General Discussions – ToRs for 2006
- 15:30 **Health Break**
- 16:00 **ToR f:** Collate and assess National reports and update the decadal mapping of harmful algal events for the IOC/ICES harmful algal database, HAE-DAT on a regional temporal and species basis (Country Reps)
- 17:30 Election of a New Chair for the next 3 years  
Meeting location for 2006
- 17:45 Report writing
- 18:30 Adjourn for the day

#### Wednesday, 6 April

- 08:30 **ToR c):** discuss new findings that pertain to HAB dynamics, and define the main processes (Joe Silke, Per Anderson, Louis Peperzak (3), others.....)
- 10:30 **Health break**
- 11:00 Terms of reference 2006
- 12:30 **LUNCH**
- 13:30 **ToR b:** consider the status of knowledge concerning biologically active specific chemicals, their chemical nature, presence and production in algae and their effects on individuals and population dynamics, as well as their impacts on ecosystems (Allan Cembella, Tonie Castberg, others .....)
- 14:30 Report writing
- 15:30 **Health Break**
- 16:00 Report Writing; subgroup and plenary sessions to be decided on ‘ad hoc’ basis
- 17:00 Adoption of sections of the report
- 18:30 Adjourn for the day

#### Thursday, 7 April

- 08:30 Adoption of all sections of the report
- 10:00 **Health Break**
- 10:15 Finalize ToRs for 2006
- 12:00 Meeting adjournment .....LUNCH
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